

CLAIMS

What is claimed is:

1. A composition comprising an isolated polynucleotide encoding a protein having TNF-R1-DD ligand protein activity.

5 2. The composition of claim 1 wherein said polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

10 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2; and

15 (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

3. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

20 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3;

25 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

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5 4. A composition of claim 1 wherein said polynucleotide is operably linked
to an expression control sequence.

5. A host cell transformed with a composition of claim 4.

10 6. The host cell of claim 5, wherein said cell is a mammalian cell.

7. A process for producing an TNF-R1-DD ligand protein, which
comprises:

15 (a) growing a culture of the host cell of claim 5 in a suitable culture
medium; and

(b) purifying the TNF-R1-DD ligand protein from the culture.

8. A composition comprising a protein having TNF-R1-DD ligand protein
activity.

20 9. The composition of claim 8 wherein said protein comprises an amino
acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2; and

(b) fragments of the amino acid sequence of SEQ ID NO:2;
25 said protein being substantially free from other mammalian proteins.

10. The composition of claim 8 wherein said protein comprises an amino
acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4; and

30 (b) fragments of the amino acid sequence of SEQ ID NO:4;
said protein being substantially free from other mammalian proteins.

11. The composition of claim 8 wherein said protein comprises an amino
acid sequence selected from the group consisting of:

35 (a) the amino acid sequence of SEQ ID NO:6; and

(b) fragments of the amino acid sequence of SEQ ID NO:6;

5 said protein being substantially free from other mammalian proteins.

12. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.

10 13. A composition comprising an antibody which specifically reacts with the TNF-R1-DD ligand protein of claim 8.

14. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

- 15 (a) combining an TNF-R death domain protein with a composition of claim 8, said combination forming a first binding mixture;
- (b) measuring the amount of binding between the TNF-R death domain protein and the TNF-R1-DD ligand protein in the first binding mixture;
- (c) combining a compound with the TNF-R death domain protein
- 20 and an TNF-R1-DD ligand protein to form a second binding mixture;
- (d) measuring the amount of binding in the second binding mixture; and
- (e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

25 wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the amount of binding of the second binding mixture occurs.

15. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

- 30 (a) the amino acid sequence of SEQ ID NO:2;
- (b) fragments of the amino acid sequence of SEQ ID NO:2;
- (c) the amino acid sequence of SEQ ID NO:4;

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5 22. The composition of claim 21 further comprising a pharmaceutically acceptable carrier.

 23. A method of preventing or ameliorating an inflammatory condition comprising administering to a mammalian subject a therapeutically effective amount
10 of the composition of claim 22.

 24. A method of inhibiting TNF-R death domain binding comprising administering to a mammalian subject a therapeutically effective amount of the composition of claim 22.
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 25. A composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

20 26. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

 (a) transforming a cell with a first polynucleotide encoding an TNF-R death domain protein, a second polynucleotide encoding an TNF-R1-DD ligand protein, and at least one reporter gene, wherein the expression of the reporter gene is regulated by the binding of the TNF-R1-DD ligand protein
25 encoded by the second polynucleotide to the TNF-R death domain protein encoded by the first polynucleotide;

 (b) growing the cell in the presence of and in the absence of a compound; and

30 (c) comparing the degree of expression of the reporter gene in the presence of and in the absence of the compound;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the degree of expression of the reporter gene occurs.

35 27. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1, which encodes a protein having TNF-R1-DD ligand protein activity;
- 10 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;
- (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 and having TNF-R1-DD ligand protein activity;
- 15 (e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;
- (f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3, which encodes a protein having TNF-R1-DD ligand protein activity;
- 20 (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;
- (h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 and having TNF-R1-DD ligand protein activity;
- 25 (i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 2 to nucleotide 559;
- (j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5, which encodes a protein having TNF-R1-DD ligand protein activity;
- 30 (k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:6;
- (l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 and having TNF-R1-DD ligand protein activity;
- 35 (m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 57 to nucleotide 875;

5 (n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7, which encodes a protein having TNF-R1-DD ligand protein activity;

(o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:8;

10 (p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 and having TNF-R1-DD ligand protein activity; and

(q) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(p), which encodes
15 a protein having TNF-R1-DD ligand protein activity.

28. The method of claim 26 wherein the cell is a yeast cell.

29. The composition of claim 1 wherein said polynucleotide sequence is
20 selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;

25 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10; and

(e) a polynucleotide capable of hybridizing under stringent
30 conditions to any one of the polynucleotides specified in (a)-(d).

30. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID
35 NO:11 from nucleotide 2 to nucleotide 1822;

5 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

10 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

15 31. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10; and

(b) fragments of the amino acid sequence of SEQ ID NO:10; said protein being substantially free from other mammalian proteins.

20 32. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12; and

(b) fragments of the amino acid sequence of SEQ ID NO:12; said protein being substantially free from other mammalian proteins.

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33. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) fragments of the amino acid sequence of SEQ ID NO:10;

30 (c) the amino acid sequence of SEQ ID NO:12; and

(d) fragments of the amino acid sequence of SEQ ID NO:F2.

34. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

35 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

- 5 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9, which encodes a protein having TNF-R1-DD ligand protein activity;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;
- 10 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 and having TNF-R1-DD ligand protein activity;
- (e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;
- 15 (f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11, which encodes a protein having TNF-R1-DD ligand protein activity;
- (g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12; and
- 20 (h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 and having TNF-R1-DD ligand protein activity; and
- (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), which encodes
- 25 a protein having TNF-R1-DD ligand protein activity.

35. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID
- 30 NO:13 from nucleotide 3 to nucleotide 2846;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;
- 35 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14; and

5 (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

36. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

- 10 (a) the amino acid sequence of SEQ ID NO:14; and
(b) fragments of the amino acid sequence of SEQ ID NO:14;
said protein being substantially free from other mammalian proteins.

37. The method of claim 14 wherein said TNF-R1-DD ligand protein
15 comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:14; and
(b) fragments of the amino acid sequence of SEQ ID NO:14.

38. The method of claim 26 wherein the second polynucleotide is selected
20 from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;
(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13, which encodes a protein having TNF-R1-DD ligand protein activity;
25 (c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;
(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 and
30 having TNF-R1-DD ligand protein activity; and
(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d), which encodes a protein having TNF-R1-DD ligand protein activity.

39. The composition of claim 1 wherein said polynucleotide sequence is
35 selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 326 to nucleotide 5092;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:15;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein
- 10 comprising the amino acid sequence of SEQ ID NO:16;
- (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:16; and
- (e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

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40. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16; and
 - (b) fragments of the amino acid sequence of SEQ ID NO:16;
- 20 said protein being substantially free from other mammalian proteins.

41. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16; and
- 25 (b) fragments of the amino acid sequence of SEQ ID NO:16.

42. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID
- 30 NO:15 from nucleotide 326 to nucleotide 5092;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:15, which encodes a protein having TNF-R1-DD ligand protein activity;
- (c) a polynucleotide encoding an TNF-R1-DD ligand protein
- 35 comprising the amino acid sequence of SEQ ID NO:16;

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5 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 and having TNF-R1-DD ligand protein activity; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d), which encodes
10 a protein having TNF-R1-DD ligand protein activity.

43. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID
15 NO:17 from nucleotide 14 to nucleotide 2404;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:17;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:18;

20 (d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:18; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

25 44. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:18; and

(b) fragments of the amino acid sequence of SEQ ID NO:18;
said protein being substantially free from other mammalian proteins.

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45. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:18; and

(b) fragments of the amino acid sequence of SEQ ID NO:18.

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